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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313 on September 29, 2004.

Frank C. Eisenschenk

Frank C. Eisenschenk, Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. SPO-103
Patent No. 6,719,976

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Toshio Sone, Akinori Kume, Kazuo Dairiki, Akiko Iwama, Kohsuke Kino
Issued	:	April 13, 2004
Patent No.	:	6,719,976 B1
For	:	Peptide-Based Immunotherapeutic Agent for Treating Allergic Diseases

Mail Stop CERTIFICATE OF CORRECTIONS BRANCH
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Patent Should Read:**
Title Page, Item 56, References Cited, Other Publications, Left Column, 4th reference, Taniai *et al*: "Taniai, Madoka, Shunsaku, Ando, Mitsuko Usui, Masashi Kurimoto et al. (Nov. 1988) "N-terminal amino acid sequence of a major allergen of Japanese ceder pollen (Cry J 1)"60 *FEBS LETTERS 239(2):329-332.*"	Information Disclosure Statement dated May 20, 1999, Reference AR, Taniai *et al*: -- Taniai, Madoka, Shunsaku Ando, Mitsuko Usui, Masashi Kurimoto et al. (Nov. 1988) "N-terminal amino acid sequence of a major allergen of Japanese cedar pollen (*Cry j* 1)" *FEBS LETTERS 239(2):329-332.* --
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Title page, Item 57, Abstract, Line 5: "multi-epitope peptide can treat a wide"	Preliminary Amendment dated July 30, 1999, Abstract, Line 7: --multi-epitope peptide can prevent and treat a wide--

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Patent Reads:	**Application Reads:**
Column 1, Line 49: "ßchain"	Page 2, Line 16: -- ß-chain --
Column 3, Line 8: "77-36"	Page 5, Line 23: --77-86--
Column 4, Line 39: "for each, patient"	Page 9, Lines 8-9: --for each patient--
Column 4, Line 60: "class It molecules"	Page 10, Line 3: --class II molecules--
Column 5, Line 8: "DPB1*10.501"	Page 10, Line 16: --DPB1*0501--
Column 7, Line 62: "(DRB1*140)"	Page 17, Line 4: --(DRB1*1405)--
Column 7, Line 64: "of BCGA antigen"	Page 17, Line 5: --of BCGa antigen.--
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Column 8, Lines 54: "Brief Description of the Sequences" is missing	Page 19, Lines 4-6: --Brief Description of the Sequences SEQ ID NOs: 1-174 provide amino acid sequences for various peptides disclosed herein. SEQ ID NOs: 15 through 174 are found in Figures 1, 2, 7, 15, 17, and 18.--
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Column 14, Line 14: "4-methylumbelliferyl-, ß-D-galacto-pyranoside/0.01 M"	Page 31, Lines 9-10: --4-methylumbelliferyl-ß-D-galacto-pyranoside/0.01 M --
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Column 15, Lines 38-39: "proliferation scintillation counter."	Page 34, Line 9: --proliferation response (Fig. 10).--
Column 15, Lines 40-43: Same as preceding paragraph.	Page 34, Lines 6-9: This paragraph should not be duplicated.
Column15, Lines 40-43: "The peripheral lymphocytes from five out of six patients showed proliferation response to the multi-epitope peptide. The peripheral lymphocytes from one patient and two healthy subjects showed no proliferation response (FIG. 10)."	Page 34, Lines 10-14: --The proliferation response of peripheral lymphocytes began to occur with stimulation of 01.µg/ml of the multi-epitope peptide and increased dose-dependently. Based on the results, the concentration of the multi-epitope peptide required for inducing T cell proliferation response in vitro is at least 10µg/ml.--

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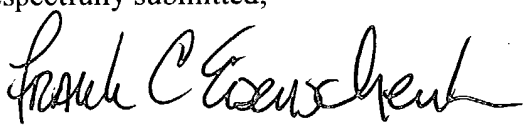
Column 18, Line 48: "N. Hei 8-15327), or "	Page 41, Line 6: --No. Hei 8-153527), or--
Patent Reads:	**Patent Should Read:**
Column 20, Line 30, Claim 3: "A peptide-based immnunotherapeutic"	Amendment Dated September 22, 2003, Claim 51 (re-numbered as Claim 3): -- A peptide-based immunotherapeutic --
Column 20, Line 34, Claim 4: "carrier or diluent or diluent and"	Amendment Dated September 22, 2003, Claim 71 (re-numbered as Claim 4): --carrier or diluent and--

A copy of pages 2, 5, 9, 10, 17, 19, 21, 23-28, 30-31, 34, 36, and 41 of the specification as filed, a copy of the Information Disclosure Statement dated May 20, 1999, a copy of the Preliminary Amendment dated July 30, 1999, and a copy of the Amendment dated September 22, 2003, which support Applicants' assertion of errors on the part of the Patent Office, accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



Frank C. Eisenschenk, Ph.D.
Patent Attorney
Registration No. 45,332
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: P.O. Box 142950
Gainesville, FL 32614-2950

FCE/ssa
Attachments: Certificate of Correction (in duplicate)
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INVENTORS : Toshio Sone, Akinori Kume, Kazuo Dairiki, Akiko Iwama, Kohsuke Kino

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 12:
Line 53 "Identifying the Type of T Cell Clone"

should read

--Identifying the Th Type of T Cell Clone--

Column 13:
Line 54 "C.A.#1. a-Arg-Arg-b-Arg-Arg-c-Arg-Arg-e-Arg-Arg"

should read

--C.A.#1. a-Arg-Arg-b-Arg-Arg-c-Arg-Arg-d-Arg-Arg-e --

Column 14:
Line 14 "4-methylumbelliferyl-, ß-D-galacto-pyranoside/0.01 M"

should read

--4-methylumbelliferyl-ß-D-galacto-pyranoside/0.01 M --

MAILING ADDRESS OF SENDER:
Saliwanchik, Lloyd & Saliwanchik
P.O. Box 142950
Gainesville, FL 32614-2950

PATENT NO. 6,719,976 B1

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FORM PTO-1050 (REV. 3-75) UNITED STATES PATENT AND TRADEMARK OFFICE

OCT 13 2004

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,719,976 B1

DATED : April 13, 2004

INVENTORS : Toshio Sone, Akinori Kume, Kazuo Dairiki, Akiko Iwama, Kohsuke Kino

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 14:
Line 16 "NaN, and "

should read

--NaN_3 and--

Column 15:
Lines 38-39 "proliferation scintillation counter."

should read

--proliferation response (Fig. 10).--

Column 15:
Lines 40-43 "This paragraph is the same as the preceding paragraph."

--This paragraph should not be repeated.--

MAILING ADDRESS OF SENDER:
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P.O. Box 142950
Gainesville, FL 32614-2950

PATENT NO. 6,719,976 B1

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FORM PTO-1050 (REV. 3-75) UNITED STATES PATENT AND TRADEMARK OFFICE

OCT 13 2004

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,719,976 B1

DATED : April 13, 2004

INVENTORS : Toshio Sone, Akinori Kume, Kazuo Dairiki, Akiko Iwama, Kohsuke Kino

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column15:
Lines 40-43 "The peripheral lymphocytes from five out of six patients showed proliferation response to the multi-epitope peptide. The peripheral lymphocytes from one patient and two healthy subjects showed no proliferation response (FIG. 10)."

should read

--The proliferation response of peripheral lymphocytes began to occur with stimulation of 01.μg/ml of the multi-epitope peptide and increased dose-dependently. Based on the results, the concentration of the multi-epitope peptide required for inducing T cell proliferation response in vitro is at least 10μg/ml.--

Column 16:
Line 45 "spleen cells ($5x10^6$ cells) were cultured"

should read

--spleen cells were collected from three mice and mixed together. The spleen cells ($5x10^6$ cells) --

Column 18:
Line 48 "N. Hei 8-15327), or "

should read

--No. Hei 8-153527), or--

MAILING ADDRESS OF SENDER:
Saliwanchik, Lloyd & Saliwanchik
P.O. Box 142950
Gainesville, FL 32614-2950

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FORM PTO-1050 (REV. 3-75) UNITED STATES PATENT AND TRADEMARK OFFICE

OCT 13 2004

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,719,976 B1

DATED : April 13, 2004

INVENTORS : Toshio Sone, Akinori Kume, Kazuo Dairiki, Akiko Iwama, Kohsuke Kino

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 20:
Line 30, Claim 3 "A peptide-based immnunotherapeutic"

should read

-- A peptide-based immunotherapeutic --

Column 20:
Line 34, Claim 4 "carrier or diluent or diluent and"

should read

--carrier or diluent and--

MAILING ADDRESS OF SENDER:
Saliwanchik, Lloyd & Saliwanchik
P.O. Box 142950
Gainesville, FL 32614-2950

PATENT NO. 6,719,976 B1

No. of add'l. copies
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FORM PTO-1050 (REV. 3-75) UNITED STATES PATENT AND TRADEMARK OFFICE

OCT 13 2004

INFORMATION DISCLOSURE STATEMENT
Patent Application
Docket No. SPO-103
Serial No. 09/142,524
Art Unit: 1646

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	:	1646
Applicant(s)	:	Toshio Sone, Akinori Kume, Kazuo Dairiki, Akiko Iwama, Kohsuke Kino
Serial No.	:	09/142,524
Filed	:	September 9, 1998
For	:	Peptide-based Immunotherapeutic Agent For Treating Allergic Diseases

Assistant Commissioner for Patents
Washington, D.C. 20231

INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR §§1.97 AND 1.98

Sir:

In accordance with 37 CFR §1.56, the references listed on the attached form PTO-1449 are being brought to the attention of the Examiner for consideration in connection with the examination of the above-identified patent application. Copies of the cited documents are enclosed.

The Applicants respectfully assert that the substantive provisions of 37 CFR §§1.97 and 1.98 are met by the foregoing statement.

Respectfully submitted,

David Saliwanchik

David R. Saliwanchik
Patent Attorney
Registration No. 31,794
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address : 2421 N.W. 41st Street, Suite A-1
Gainesville, FL 32606-6669

DRS/la
Enclosures: as stated above

Form PTO-1449 (REV. 7-80)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTY. DOCKET NO. SPO-103	SERIAL NO. 09/142,524
	INFORMATION DISCLOSURE STATEMENT *(Use several sheets if necessary)*	APPLICANT(S): Toshio Sone, Akinori Kume, Kazuo Dairiki, Akiko Iwama, Kohsuke Kino	
		FILING DATE September 9, 1998	GROUP ART UNIT 1646

U.S. PATENT DOCUMENTS

*EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA						
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
	AL	9 4 0 1 5 6 0	1/20/94	PCT				
	AM							
	AN							
	AO							
	AP							

OTHER DOCUMENTS *(Including Author, Title, Date, Pertinent Pages, Etc.)*

	AR		Taniai, Modoka, Shunsaku Ando, Mitsuko Usui, Masashi Kurimoto *et al.* (November 1988) "N-terminal amino acid sequence of a major allergen of Japanese cedar pollen (*Cry j* I)" *FEBS LETTERS 239(2):329-332.*
	AS		Rogers, Bruce L., Julian F. Bond, Sandra J. Craig *et al.* (1994) "Potential Therapeutic Recombinant Proteins Comprised Of Peptides Containing Recombined T Cell Epitopes" *Molecular Immunology* 31(13):955-966.
	AT		Komiyama, Naoki, Toshio Sone, Kimiko Shimizu, Keiko Morikubo, Hohsuke Kino (June 15, 1994) "cDNA Cloning And Expression of *Cry j* II, the Second Major Allergen of Japanese Cedar Pollen" *Biochemical and Biophysical Research Communications* 201(2):1021-1028.
	AU		Higgins, Julie A., Christopher J. Thorpe, John D. Hayball, Robyn E. O'Hehir, Jonathan R. Lamb (May 1994) "Overlapping T-cell epitopes in the group I allergen of *Dermatophagoides* species restricted by HLA-DP and HLA-DR class II molecules" *J. Allergy Clin. Immunol.* pp. 891-899.
	AV		Matsunaga, Youichi, Toshiji Saibara, Hiroshi Kido, Nobuhiko Katunuma (June 1993) "Participation of cathepsin B in processing of antigen presentation to MHC class II" *FEBS LETTERS* 324(3):325-330.

EXAMINER	DATE CONSIDERED

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Form PTO-1449 (REV. 7-80)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTY. DOCKET NO. SPO-103	SERIAL NO. 09/142,524
INFORMATION DISCLOSURE STATEMENT *(Use several sheets if necessary)*		APPLICANT(S): Toshio Sone, Akinori Kume, Kazuo Dairiki, Akiko Iwama, Kohsuke Kino	
		FILING DATE September 9, 1998	GROUP ART UNIT 1646

U.S. PATENT DOCUMENTS

*EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA						
	AB						
	AC						
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FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
	AL							
	AM							
	AN							
	AO							
	AP							

OTHER DOCUMENTS *(Including Author, Title, Date, Pertinent Pages, Etc.)*

	AR		LaSalle, Janine M., Paul J. Tolentino, Gordon J. Freeman, Lee M. Nadler, David A. Hafler (July 1992) "Early Signaling Defects in Human T Cells Anergized by T Cell Presentation of Autoantigen" *J. Exp. Med.* 176:177-186.
	AS		Simons, F. Estelle R., Mie Imada, Yan Li, Wade T.A. Watson, Kent T. HayGlass (1996) "Fel d 1 peptides: effect on skin tests and cytokine synthesis in cat-allergic human subjects" *International Immunology* 8(12):1937-1945.
	AT		Brinker, Thomas J., Mei-Chang Kuo, Kathleen M. Keating, Bruce L. Rogers, Julia L. Greenstein (August 1993) "Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d I" *Proc. Natl. Acad. Sci. USA* 90:7608-7612.
	AU		Sakaguchi, M., S. Inouye, M. Taniai, S. Ando, M. Usui, T. Matuhasi (1990) "Identification of the second major allergen of Japanese cedar pollen" *Allergy* 45:309-312.
	AV		Norman, Philip S., John L. Ohman, Jr., A.A. Long, Peter S. Creticos *et al.* (1996) "Treatment of Cat Allergy with T-cell Reactive Peptides" *Am J Respir Crit Care Med* 154:1623-1628.

EXAMINER	DATE CONSIDERED

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Form PTO-1449 (REV. 7-80)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTY. DOCKET NO. SPO-103	SERIAL NO. 09/142,524
INFORMATION DISCLOSURE STATEMENT *(Use several sheets if necessary)*		APPLICANT(S): Toshio Sone, Akinori Kume, Kazuo Dairiki, Akiko Iwama, Kohsuke Kino	
		FILING DATE September 9, 1998	GROUP ART UNIT 1646

U.S. PATENT DOCUMENTS

*EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
	AA						
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	AC						
	AD						
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	AI						
	AJ						
	AK						

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
	AL							
	AM							
	AN							
	AO							
	AP							

OTHER DOCUMENTS *(Including Author, Title, Date, Pertinent Pages, Etc.)*

	AR		Wallner, B.P., M.L. Gefter (1994) "Immunotherapy with T-cell-reactive peptides derived from allergens" *Allergy* 49:302-308.
	AS		Yasueda, Hiroshi, Yasuo Yui, Takaharu Shimizu, Takao Shida (1983) "Isolation and partial characterization of the major allergen from Japanese cedar (*Cryptomeria japonica*) pollen" *J. Allergy Clin. Immunol.* 71(1) part 1:77-86.
	AT		Hashimoto, M., H. Nigi, M. Sakaguchi, S. Inouye *et al.* (1995) "Sensitivity to two major allergens (*Cry j* I and *Cry j* II) in patients with Japanese cedar (*Cryptomeria japonica*) pollinosis" *Clinical and Experimental Allergy* 25:848-852.
	AU		Rammensee, Hans-Georg, Thomas Friede, Stefan Stevanovic (1995) "MHC ligands and peptide motifs: first listing" *Immunogenetics* 41:178-228.
	AV		

EXAMINER	DATE CONSIDERED

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to:
Assistant Commissioner for Patents
Washington, D.C. 20231 July 30, 1999.

David Saliwanchik

David R. Saliwanchik, Patent Attorney

PRELIMINARY AMENDMENT
Patent Application
Docket No. SPO-103
Serial No. 09/142,524
Art Unit: 1644

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	M. Dibrino
Art Unit	:	1644
Applicants	:	Toshio Sone, Akinori Kume, Kazuo Dairiki, Akiko Iwama, Kohsuke Kino
Serial No.	:	09/142,524
Filed	:	January 4, 1999
For	:	Peptide-based Immunotherapeutic Agent For Treating Allergic Diseases

Assistant Commissioner for Patents
Washington, D.C. 20231

COPY

PRELIMINARY AMENDMENT

Sir:

Please amend the above-identified application as follows, in order to comply with the requirements of 37 CFR 1.823:

In the Specification:

Please cancel existing pages 45-46. Please renumber page 49 (abstract) as page 47. After page 47, please insert new pages 1-3 of the Sequence Listing.

In the Claims

Please renumber claim pages 47-48 as pages 45-46.

Remarks

This amendment is made to conform the application with the provisions of 37 CFR §§1.821 through 1.825. I hereby certify that no new material is being added by this submission.

Respectfully submitted,

David Saliwanchik

David R. Saliwanchik
Patent Attorney
Registration No. 31,794
Phone No.: 352-375-8100
Address : 2421 N.W. 41st Street
Suite A-1
Gainesville, FL 32606

DRS/la
Attachment: New pages of Sequence Listing 1-2.

Sequence Listing

SEQ ID NO:1

SEQUENCE LENGTH: 80

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION:

MKVTVAFNQF GPNRRVFIKR VSNVIIHGRR IDIFASKNFH 40

LQKNTIGTGR RISLKLTSGK IASRRVDGII AAYQNPASWK 80

SEQ ID NO:2

SEQUENCE LENGTH: 105

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION:

MKVTVAFNQF GPNRRVFIKR VSNVIIHGRR IDIFASKNFH 40

LQKNTIGTGR RWKNNRIWLQ FAKLTGFTLM GRRLKMPMYI 80

AGYKTFDGRR VDGIIAAYQN PASWK 105

SEQ ID NO:3

SEQUENCE LENGTH: 134

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

cancelled & replaced by July 30, 1999 Prelim. Amend. jgj

MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION:

MKVTVAFNQF GPNRRVFIKR VSNVIIHGRR IDIFASKNFH 40

LQKNTIGTGR RWKNNRIWLQ FAKLTGFTLM GRRPLWIIFS 80

GNMNIKLKMP MYIAGYKTFD GRRAEVSYVH VNGAKFIRRV 120

DGIIAAYQNP ASWK 134

SEQ ID NO:4

SEQUENCE LENGTH: 31

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION:

IFSKNLNIKL NMPLYIAGNK RRFIKRVSNV I 31

SEQ ID NO:5

SEQUENCE LENGTH: 31

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE DESCRIPTION:

SSGKNEGTNI YNNNEAFKVE RRFIKRVSNV I 31

cancelled by & replaced July 30, 1999 Prelim. Amend. jaj

ABSTRACT

The present invention provides a monomolecular multi-epitope peptide prepared by binding T cell epitope regions derived from different allergen molecules with each other. A peptide-based immunotherapeutic agent containing an effective amount of the multi-epitope peptide can prevent and treat a wide range of allergic diseases.

SEQUENCE LISTING

<110> Sone, Toshio
Kume, Akinori
Kairiki, Kazuo
Iwama, Akiko
Kino, Kohsuke

<120> Peptide-based Immunotherapeutic Agent For Treating Allergic Diseases

<130> Docket No. SPO-103

<140> 09/142,524

<141> 1999-01-04

<150> 8/80/702

<151> 1996-03-10

<150> PCT/JP97/00740

<151> 1997-03-10

<160> 5

<170> PatentIn Ver. 2.0

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<213> Unknown

<220>
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<400> 1

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 1               5                  10                  15

Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly Arg Arg Ile Asp
            20                  25                  30

Ile Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr
        35                  40                  45

Gly Arg Arg Ile Ser Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser Arg
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 65                  70                  75                  80

<210> 2
<211> 105
<212> PRT
<213> Unknown

<220>
<223> Description of Unknown Organism:peptide
<400> 2

Met Lys Val Thr Val Ala Phe Asn Gln Phe Gly Pro Asn Arg Arg Val
  1               5                  10                  15

Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly Arg Arg Ile Asp
             20                  25                  30

Ile Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr
         35                  40                  45

Gly Arg Arg Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu
     50                  55                  60

Thr Gly Phe Thr Leu Met Gly Arg Arg Leu Lys Met Pro Met Tyr Ile
 65                  70                  75                  80

Ala Gly Tyr Lys Thr Phe Asp Gly Arg Arg Val Asp Gly Ile Ile Ala
                 85                  90                  95

Ala Tyr Gln Asn Pro Ala Ser Trp Lys
            100                 105



<210> 3
<211> 134
<212> PRT
<213> Unknown

<220>
<223> Description of Unknown Organism:peptide

<400> 3

Met Lys Val Thr Val Ala Phe Asn Gln Phe Gly Pro Asn Arg Arg Val
  1               5                  10                  15

Phe Ile Lys Arg Val Ser Asn Val Ile Ile His Gly Arg Arg Ile Asp
             20                  25                  30

Ile Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr
         35                  40                  45
```

```
Gly Arg Arg Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu
     50                  55                  60
Thr Gly Phe Thr Leu Met Gly Arg Arg Pro Leu Trp Ile Ile Phe Ser
 65                  70                  75                  80

Gly Asn Met Asn Ile Lys Leu Lys Met Pro Met Tyr Ile Ala Gly Tyr
                85                  90                  95

Lys Thr Phe Asp Gly Arg Arg Ala Glu Val Ser Tyr Val His Val Asn
            100                 105                 110

Gly Ala Lys Phe Ile Arg Arg Val Asp Gly Ile Ile Ala Ala Tyr Gln
        115                 120                 125

Asn Pro Ala Ser Trp Lys
    130
```

<210> 4
<211> 31
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<220>
<223> Description of Unknown Organism:peptide

<400> 4

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Ile Phe Ser Lys Asn Leu Asn Ile Lys Leu Asn Met Pro Leu Tyr Ile
  1               5                  10                  15

Ala Gly Asn Lys Arg Arg Phe Ile Lys Arg Val Ser Asn Val Ile
            20                  25                  30
```

<210> 5
<211> 31
<212> PRT
<213> Unknown
<220>
<223> Description of Unknown Organism:peptide

<400> 5

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  1               5                  10                  15

Phe Lys Val Glu Arg Arg Phe Ile Lys Arg Val Ser Asn Val Ile
            20                  25                  30
```

Application No.: 09/142,524

NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825 for the following reason(s)

☑ 1. This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to these regulations, published at 1114 OG 29, May 15, 1990 and at 55 FR 18230, May 1, 1990.

☐ 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 C.F.R. 1.821(c).

☑ 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 C.F.R. 1.821(e).

☐ 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 C.F.R. 1.822 and/or 1.823, as indicated on the attached copy of the marked -up "Raw Sequence Listing."

☐ 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A Substitute computer readable form must be submitted as required by 37 C.F.R. 1.825(d).

☐ 6. The paper copy of the "Sequence Listing" is not the same as the computer readable from of the "Sequence Listing" as required by 37 C.F.R. 1.821(e).

☐ 7. Other: _____

Applicant Must Provide:

☑ An initial computer readable form (CRF) copy of the "Sequence Listing".

☐ An initial paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification.

☑ A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d).

For questions regarding compliance to these requirements, please contact:

For Rules Interpretation, call (703) 308-4216
For CRF Submission Help, call (703) 308-4212
For PatentIn software help, call (703) 308-6856

PLEASE RETURN A COPY OF THIS NOTICE WITH YOUR RESPONSE

COPY FOR [ ] File [✓] Applicant

I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on the date shown below:

September 22, 2003

Frank C Eisenschenk

Frank C. Eisenschenk, Ph.D., Patent Attorney

AMENDMENT UNDER 37 C.F.R. § 1.116
Examining Group 1644
Patent Application
Docket No. SPO-103
Serial No. 09/142,524

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Marianne N. DiBrino, Ph.D.
Art Unit	:	1644
Applicants	:	Toshio Sone, Akinori Kume, Kazuo Dairiki, Akiko Iwama, Kohsuke Kino
Serial No.	:	09/142,524
Filed	:	September 9, 1998
Confirm. No.	:	2300
For	:	Peptide-Based Immunotherapeutic Agent for Treating Allergic Diseases

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT UNDER 37 C.F.R. § 1.116

A Petition and Fee for a one-month Extension of Time through and including September 21, 2002, a accompanies this Amendment.

In response to the Office Action May 21, 2003, please amend the above-identified application as follows:

In the Claims

Claims 1-48 (Canceled).

Claim 49 (Previously added): A peptide-based immunotherapeutic agent comprising the amino acid sequence of SEQ ID NO: 1.

Claim 50 (Previously added): A peptide-based immunotherapeutic agent comprising the amino acid sequence of SEQ ID NO: 2.

Claim 51 (Previously added): A peptide-based immunotherapeutic agent comprising the amino acid sequence of SEQ ID NO: 3.

Claims 52-70 (Canceled).

Claim 71 (New). A method of treating cedar pollinosis comprising the administration of a composition comprising a pharmaceutically acceptable carrier or diluent and a peptide-based immunotherapeutic agent comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, and 3.

Claim 72 (New). The method according to claim 71, wherein said peptide-based immunotherapeutic agent comprises SEQ ID NO: 1.

Claim 73 (New). The method according to claim 71, wherein said peptide-based immunotherapeutic agent comprises SEQ ID NO: 2.

Claim 74 (New). The method according to claim 71, wherein said peptide-based immunotherapeutic agent comprises SEQ ID NO: 3.

Claim 75 (New). A composition comprising a pharmaceutically acceptable carrier or diluent and a peptide-based immunotherapeutic agent comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, and 3.

Claim 76. (New). The composition according to claim 75, wherein said peptide-based immunotherapeutic agent comprises SEQ ID NO: 1.

Claim 77 (New). The composition according to claim 75, wherein said peptide-based immunotherapeutic agent comprises SEQ ID NO: 2.

Claim 78 (New). The composition according to claim 75, wherein said peptide-based immunotherapeutic agent comprises SEQ ID NO: 3.

Remarks

Claims 1, 4, 5, 13, 32-34 and 48-70 were pending in the subject application. By way of this amendment, claims 1-48 and 52-70 have been canceled and claims 71-78 are newly added. Thus, claims 49-51 and 71-78 are before the Examiner for consideration. The undersigned avers that no new matter is introduced by this amendment and that support for the newly presented claims can be found in the application and claims as originally filed (see, for example, page 18, about line 15). Accordingly, entry and consideration of the amendment presented herein is respectfully requested. Favorable consideration of the pending claims is also respectfully requested.

Applicants gratefully acknowledge the Examiner's indication of allowable subject matter with respect to the subject invention and have amended the claims to facilitate the allowance of the application. New claims 71-78 have been presented to parallel previously presented claims that provided compositions comprising the allowable peptide-based immunotherapeutic agents and methods of using the same. It is respectfully submitted that the subject amendment does not raise new issues or require consideration of the claims that not previously performed.

In view of the foregoing remarks and amendments to the claims, the applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested. It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position. Applicants expressly reserve the right to pursue the invention(s) disclosed in the subject application, including any subject matter canceled or not pursued during prosecution of the subject application, in a related application.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

The applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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SUBJECT/MESSAGE: U.S. Patent Application Docket No. SPO-103
Peptide-Based Immunotherapeutic Agent for Treating Allergic Diseases
(Sone, *et al.*)
Serial No. 09/142,524; Filed September 9, 1998

ATTACHMENT: Petition and Fee for Extension of TIme Under 37 C.F.R. § 1.136(a)
Amendment Under 37 C.F.R. § 1.116

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studies focus on clarifying the initial reaction in the allergen-specific immune response, especially the mechanism of regulating a T cell-mediated allergic reaction. Initiation of an immune response to a foreign antigen including an allergen depends on antigen-presenting cells in the immune system. The antigen-presenting cells (i.e., B cells, macrophages, and dendritic cells) take up incoming foreign antigens, break them down to antigen peptides (T cell epitope peptides), put the fragments in a pocket consisting of α and β chains of major histocompatibility complex (MHC) class II molecules (HLA class II in human), display the fragments on the cell surface, and thereby present the foreign antigens to antigen-specific CD4 positive helper T cells (Th cells). An HLA class II molecule consists of DR, DQ and DP molecules. The α-chain of the DR molecule is encoded by the HLA-DRA gene, and the β-chain is encoded by the HLA-DRB1, -DRB3, -DRB4 or -DRB5 gene. The α-chain of the DQ molecule is encoded by the HLA-DQA1 gene, and the β-chain is encoded by the HLA-DQB1 gene. The α-chain of the DP molecule is encoded by the HLA-DPA1 gene, and the β-chain is encoded by the HLA-DPB1 gene. Each gene except for HLA-DRA contains many alleles. The pocket in which antigenic peptides are placed is highly polymorphic, and the structures differ slightly from each other. Because of this, the kind of antigenic peptides that bind to the pocket and are presented to T cells is restricted to that structure.

Once Th cells receive HLA class II-restricting antigen information via the T cell receptor (TCR), they are activated to

154: 1623-1628, 1996; Simons, F.E. et al.: Int. Immunol. 8: 1937-1945, 1996). Hyposensitization therapy using such a peptide carrying the major T cell epitope on the allergen molecule is called "peptide-based immunotherapy" (or "peptide-based hyposensitization therapy").

As a standard for selecting T cell epitope peptides appropriate for the peptide-based immunotherapy, a positivity index (a mean T cell stimulation index multiplied by appearance frequency) is proposed in WO 94/01560. It is also reported that in peptide design, HLA haplotypic variations in a population of patients should be covered (Wallner, B.P. & Gefter M.L.: Allergy, 49: 302-308, 1994).

Disclosure of the Invention

Generally, allergic patients have specific IgE antibodies to each of two or more allergen molecules differing from each other. For a potent allergy therapy, it is important to develop a peptide-based immunotherapeutic agent effective for these patients. However, such an immunotherapeutic agent has not yet been developed. Even the idea of such an agent has never been published in any of the above literatures. Accordingly, an objective of the present invention is to provide a peptide-based immunotherapeutic agent that is efficacious even for allergy patients sensitive to two or more different allergens.

Cedar pollen contains two major allergens, Cry j 1 (Yasueda, H. et al.: J. Allergy Clin. Immunol. 71: 77-86, 1983) and Cry j 2 (Taniai, M. et al.: FEBS Letter 239: 329-332, 1988; Sakaguchi, M. et al.: Allergy 45: 309-312, 1990). More than 90% of the patients

1201, 1994) to identify the antigenic peptides containing T cell epitopes in the Cry j 1 or Cry j 2 sequence (Figs. 1 and 2).

Next, typing is performed for HLA class II molecules which bind to these antigenic peptides.

In humans, three different molecules, regions DR, DQ, and DP, exist as gene products of the HLA class II. This suggests that differentiation of T cells would be restricted by antigen-presenting molecules DR, DQ, and DP. The T cell clones established for each patient are used to determine by which locus-derived antigen-presenting molecules the antigenic peptide of Cry j 1 or Cry j 2 is presented. They also determine whether the T cells that have received antigenic peptide information via DR, DQ or DP molecules tend to be differentiated into Th1 cells or Th2 cells. Such a typing is performed using the T cell clone established for individual patients (Figs. 3 and 4).

Figures 3 and 4 clearly show that differentiation into Th1, Th2 or Th0 of the T cells stimulated by the antigenic peptide is not restricted by a specific epitope or a specific combination of HLA molecules. In selecting a peptide for designing the multi-epitope peptide of the present invention, any peptide can be a candidate for the antigenic peptide since any T cell epitope-containing peptide can stimulate T cells.

The criteria for selecting peptides to design the multi-epitope peptide of the present invention are as follows:

(1) Peptides are selected in the order of a positivity index

(WO 94/01560) (peptides having a positivity index of 100 or more should be selected).

(2) Peptides presented on HLA class II molecules that frequently appear as antigen-presenting molecules are selected.

(3) Where there is no significant difference in the positivity index, peptides presented by restriction molecules of different types are selected to enhance the effectiveness. Specifically, in selecting a T cell epitope of an allergen that causes a certain allergic disease, the HLA haplotype in a group of patients with the allergy is first examined, and a T cell epitope restricted by an HLA haplotype whose gene frequency is high in the population to which the patient group belongs is selected. This is then the best selection that should achieve the best effect in that patient group. However, the thus-selected T cell epitope may not be effective at all in other patient groups.

Taking HLA haplotype DPB1*0501 as an example, it is assumed that this HLA haplotype is quite frequently observed in Japanese patients with a certain allergic disease, and the HLA haplotype-restricting T cell epitope is selected. The thus-selected peptide would hardly be effective for Northern American patients with the same allergy because the gene frequency of the HLA haplotype is as much as 39.0% in Japanese patients, whereas it is as little as 1.3% in white Americans and 0.8% in African Americans in Northern America. For Northern American patients, the HLA-DP restricting T cell epitope DPB1*0401 (in Northern America, 30.2% for white American patients and

with BCG inoculation revealed an increased level of Th1 type T cells (Matsushita, Sho, The 45th Japanese Association of Allergy, 836, 1995). According to Matsushita, there is a Th1 clone that is restricted by HLA-DR14 (DRB1*1405) and that recognizes 84-100 amino acid sequence (EEYLILSARDVLAVVSK) of BCGa protein. If the HLA haplotype DPA1-DPB1*0501-restricting T cell epitope that is possessed by more than 60% of Japanese population is selected (for example, Peptide No. 43 (p211-225)/KSMKVTVAFNQFGPN of Cry j 1 shown in Fig. 1), this peptide is bound to the 84-100 T cell epitope of tubercle bacillus BCGa protein restricted by DRB1*1405. It is highly likely that the thus-prepared multi-epitope peptide EEYLILSARDVLAVVSKRRMKVTVAFNQFGPN would be quite efficacious for patients with cedar pollinosis carrying haplotype DRB1*1405. The use of such a multi-epitope peptide would lead to production of Th1 lymphokines, especially IL-12, by a peptide derived from BCGa antigen. It is known in several cases in humans and mice that IL-12 has an activity contradictory to that of IL-4 and acts on T cells to induce differentiation of Th cells to Th1 (Manetti, R., et al.: J. Exp. Med., 177, 1199-1204, 1993; Wu, C., et al.: J. Immunol., 151, 1938-1949, 1993; Hsieh, C., et al.: Science, 260, 547-549, 1993). In particular, the experimental results by Manetti et al. indicate that a T cell clone specific to Der p1 antigen, a mite allergen, basically induces Th2 but induces Th1 or Th0 in the presence of IL-12. Thus, using the multi-epitope peptide prepared by joining a T cell epitope having Th1 induction activity to an allergen-reactive T cell epitope, T cells that are inherently induced

specification and the sequence listing follows the definition prescribed by IUPAC, Commission on Biochemical Nomenclature (cf., Biochemical Dictionary, 2nd ed., 1468, Table 1.1).

Brief Description of Drawings

Figure 1 shows a mean stimulation index, frequency of appearance, and a positivity index (mean stimulation index multiplied by frequency of appearance) of the cell line derived from the patients with cedar pollinosis, against Cry j 1 overlapping peptides. (SEQ ID NOS: 15-83)

Figure 2 shows a mean stimulation index, frequency of appearance, and a positivity index (mean stimulation index multiplied by frequency of appearance) of the cell line derived from the patients with cedar pollinosis, against Cry j 2 overlapping peptides. SEQ ID NOS: 84-156

Figure 3 shows the Th type of the T cell clones that recognize complexes between the Cry j 1 antigenic peptides and HLA class II molecules as well as the Th types of the HLA class II molecules.

Figure 4 shows the Th type of T cell clones that recognize complexes between the Cry j 2 antigenic peptides and HLA class II molecules as well as the Th types of the HLA class II molecules.

Figure 5 shows the results of identifying HLA class II molecules capable of binding to an antigenic peptide at the locus level (DR, DQ, and DP).

Figure 6 shows the results of identifying HLA class II molecules capable of binding to an antigenic peptide at an allelic level of each locus.

SEQ ID NOS: 162-173

Figure 15 shows core amino acid sequencing of Peptide No. 22 (p106-120) of Cry j 1.

Figure 16 shows the reactivity of T cell lines of the patients with cedar pollinosis and the patients with *hinoki* pollinosis with the multi-epitope peptide prepared by binding a cedar pollen-specific T cell epitope peptide to a Japanese cypress pollen-specific T cell epitope peptide.

SEQ ID NO: 174

Figure 17 shows the proliferation response of T cell clone PJ7-9 to an amino acid-substituted analog peptide of Cry j 1 #22 core peptide and the amount of cytokine subsequently produced.

SEQ ID NO: 174

Figure 18 shows the proliferation response of T cell clone PB10-18 to the above-described analog peptide and the amount of cytokine subsequently produced.

Best Mode for Implementing the Invention

Example 1

Identifying T cell epitope of Cry j 1 and Cry j 2 using T cell line

Peripheral lymphocytes from 18 patients with cedar pollinosis were stimulated by cedar pollen allergen Cry j 1 or Cry j 2 to establish the T cell line of each patient capable of specifically recognizing the respective allergen.

A mixture of 5×10^4 cells of the autologous B cell line treated with mitomycin C, 2μM of an overlapping peptide, and 2×10^4 cells of the T cell line was incubated for 2 days in RPMI-1640 medium supplemented with 0.2 ml of 15% serum on a 96-well culture plate. After 0.5μCi [^{3}H] thymidine was added to the medium, incubation was

to design the multi-epitope peptide. Therefore, eighteen patients were further examined with respect to the antigenic peptide which showed a stimulation index of 2 or more. A mean stimulation index of the antigenic peptide was calculated and multiplied by the rate of patients carrying the antigenic peptide (frequency in appearance) to calculate the "positivity index" which shows the predominant order for the respective epitopes (cf. WO 94/01560).

The results are shown in Figs. 1 and 2. In Cry j 1, Peptide No. 43 (p211-225) shows the highest positivity index, 679, which is followed by the second highest Peptide No. 22 with a positivity index of 578 and Peptide No. 4 with a positivity index of 373. In Cry j 2, Peptide No. 14 shows the highest positivity index (709). Peptide No. 38 with a positivity index of 680 and Peptide No. 48 with a positivity index of 370 then follow. One antigenic peptide having a high positivity index may be selected and used for the peptide-based immunotherapy. However, even for the highest appearance frequency, the effect can be theoretically expected in only 72% of the patients, and the actual efficiency would be lower. To increase the efficiency, it is necessary to use numerous T cell epitopes in combination. In this case, T cell epitopes with a high positivity index are chosen as candidates. However, just using epitopes with a high positivity index alone cannot increase the efficiency if HLA class II molecules presenting these epitopes as antigens are the same. It is thus necessary to identify the type of HLA class II molecules presenting T cell epitope peptides.

Example 2

Identifying T cell epitope peptide recognized by T cell clone

Two patients, Patient B (PB) and Patient J (PJ), who recognize Peptide Nos. 43 and 22 showing a high positivity index in Cry j 1 and three patients, PB, Patient C (PC), and Patient R (PR), who recognize Peptide Nos. 14, 38, 48, and 69 showing a high positivity index in Cry j 2 were selected from the eighteen patients with cedar pollinosis. Peripheral lymphocytes from these patients with cedar pollinosis were stimulated by Cry j 1 or Cry j 2 to establish T cell clones capable of recognizing Cry j 1 or Cry j 2. The types of HLA class I and class II molecules of the four patients are shown below.

PB: A2/24 - B39/55 - Cw7/w3 - DRB1*1501/0901 - DRB4*0101- DRB5*0101, DQA1*0102/0301 - DQB1*0602/0303 - DPA1*0101/0101 - DPB1*0501/0201;

PJ: A24/- - B61/51 - Cw3/- - DRB1*1501/0802 - DRB5*0101, DQA1*0102/0401 - DQB1*0602/0402 - DPA1*-/- - DPB1*0501/0402;

PC: A-2/2 - B54/51 - Cw1/-, DRB1*0405/1501 - DRB4*0101 - DRB5*0101 - DQA1*0301/0102 - DQB1*0401/0602 - DPA1*0202/0202 - DPB1*0201/0501;

PR: A-11/- - B60/35 - Cw7/w3 - DRB1*0901/1501 - DRB4*0101 - DRB5*0101 - DQA1*0301/0102 - DQB1*0303/0602 - DPA1*01/0202 - DPB1*0201/0201.

Thirty-five T cell clones in total that specifically

recognize Cry j 1 were established from the peripheral lymphocytes derived from PB, and 14 similar T cell clones from PJ. Likewise, 31 T cell clones, 10 T cell clones, and 17 T cell clones in total that specifically recognize Cry j 2 were established from the peripheral lymphocytes derived from PB, PC and PR, respectively. Since these T cell clones were all $CD3^+$, $CD4^+$, $CD8^-$, $TCR\alpha\beta^+$ and $TCR\gamma\delta^-$, the restriction molecules were found to be HLA class II molecules. A mixture of 5×10^4 cells of the autologous B cell line previously treated with mitomycin C, 2 μM of an overlapping peptide, and 2×10^4 cells of the T cell clone was incubated for 2 days in RPMI-1640 medium supplemented with 0.2 ml of 15% serum on a 96-well micro culture plate. After 0.5 μCi [^{3}H] thymidine was added to the medium, incubation was continued for a further 18 hours. After the cells were harvested on a glass filter using a cell harvester, the level of [^{3}H] thymidine taken up into the cells was determined using a liquid scintillation counter. By this procedure, the T cell epitope recognized by each of the T cell clones was identified.

In the T cell clones that recognized Cry j 1, 69% (34/49) showed a proliferation response by stimulation with the peptides and, as a result, the epitopes were identified. Similarly, the antigenic peptide could be identified in 69% (40/58) out of the T cell clones which recognized Cry j 2. The T cell clones capable of specifically recognizing Cry j 1 recognized Peptide Nos. 4, 13, 19, 22, 30, 31, 39, 43, 51, and 66, and the T cell clone capable of specifically recognizing Cry j 2 recognized Peptide Nos. 4, 8, 14, 17, 31, 37,

38, 48, 65, 66, 68, 69, and 70. The results are summarized in Figs. 3 and 4.

Example 3

Identifying HLA class II restriction molecules at the locus level

HLA class II restriction molecules were identified at the locus level by adding a monoclonal antibody capable of specifically reacting with DR, DQ or DP of HLA class II molecules to the proliferation response system of the T cell clones established in Example 2, thereby inhibiting the proliferation response of T cells.

A mixture of 2 x 10^4 cells of the autologous B cell line previously treated with mitomycin C; 2μM of an overlapping peptide; 3μg/ml of anti-DR, -DQ or -DP monoclonal antibody (manufactured by Becton Dickinson Inc.); and 2 x 10^4 cells of the T cell clone was incubated for 2 days in RPMI-1640 medium supplemented with 0.2 ml of 15% serum on a 96-well micro culture plate. After 0.5μCi [^{3}H] thymidine was added to the medium, incubation was continued for a further 18 hours. After the cells were harvested on a glass filter using a cell harvester, the level of [^{3}H] thymidine taken up into the cells was determined using a liquid scintillation counter. The results shown in Fig. 5 indicate that the restriction molecule of the Cry j 1 p106-120, Cry j 2 p66-80 and Cry j 2 p186-200 peptides was DR; that of the Cry j 2 p341-355 peptide was DQ; and that of the Cry j 1 p211-225 and Cry j 2 p181-195 was DP. The restriction molecules of other T cell clones were analyzed in the same manner (cf. Figs. 3 and 4).

Example 4

Identifying the HLA class II restriction molecules

HLA class II restriction molecules can be identified using the T cell clones whose restriction molecules were identified at the HLA class II locus level and, as antigen-presenting cells, mouse L-cells transfected with each type for DR and B cell line having the same haplotype for DQ or DP.

A mixture of 5×10^4 mouse L cells previously treated with mitomycin C or the B cell line coincident in haplotype; 2 μM of an overlapping peptide; 3 μg/ml of anti-DR, -DQ or -DP monoclonal antibody (manufactured by Becton-Dickinson Inc.); and 2×10^4 cells of the T cell clone was incubated for 2 days in RPMI-1640 medium supplemented with 0.2 ml of 15% serum on a 96-well micro culture plate. After 0.5 μCi [^{3}H] thymidine was added to the medium, incubation was continued for a further 18 hours. After the cells were harvested on a glass filter using a cell harvester, the level of [^{3}H] thymidine taken up into the cells was determined using a liquid scintillation counter.

The restriction molecules can be identified by observing the proliferation response of the T cell clones. The Cry j 1 p106-120 peptide-presenting restriction molecule was DRB5*0101, the Cry j 1 p211-225 peptide-presenting restriction molecule was DPA1*0101 - DPB1*0501, the Cry j 2 p66-80 peptide-presenting restriction molecule was DRB5*0101, the Cry j 2 p181-195 peptide-presenting restriction molecules was DPA1*0101 - PDB1*0201, the Cry j 2 p186-200

peptide-presenting restriction molecules was DRB4*0101, and the Cry j 2 p341-355 peptide-presenting restriction molecules was DQA1*0102 - DQB1*0602 (Fig. 6). The results obtained with the other epitope sites are shown in Figs. 3 and 4.

Example 5

Identifying the Th type of T cell clone

Th2 cells are considered to participate in the development of allergy. The current level of investigations has not completely clarified if differentiation of T cells into Th1 or Th2 cells is restricted, after antigen stimulation, by a specific epitope peptide or on a HLA class II locus level. When Th2 cells are predominantly induced after stimulation with a peptide, it is highly likely that administration of the peptide will worsen the cedar pollinosis. The T cell clones prepared in Example 2 were stimulated with the epitope peptide recognized by T cells. Th type was determined by measuring the amount of IL-2, IL-4, and IFN-γ produced.

A mixture of 1×10^5 cells of the autologous B cell line previously treated with mitomycin C, 2μM of the epitope peptide, and 5×10^5 cells of the T cell clone was incubated for 24 hours in RPMI-1640 medium supplemented with 1 ml of 10% human serum on a 24-well micro culture plate. The cells were precipitated by centrifugation to obtain the culture supernatant. IL-2, IL-4, and IFN-γ in the supernatant were determined using the respective ELISA kits commercially available [for IL-2, manufactured by R & D Inc.; for IL-4, manufactured by Medgenics Inc.; and for IFN-γ, manufactured

finding, the peptides shown in Fig. 7 were selected from the T cell epitopes of Cry j 1 and Cry j 2.

Peptides a and b shown in Fig. 7 correspond respectively to Peptide Nos. 43 and 22 of Cry j 1 shown in Fig. 1, Peptide c corresponds to No. 14 of Cry j 2 shown in Fig. 2, and Peptides d and e respectively consist of a part of the amino acids 37-38 and 69-71 of Cry j 2 shown in Fig. 2.

These six peptides were joined to each other in tandem to prepare the multi-epitope peptide of the present invention. In this case, the two peptides a and b were joined in the order of a and then b; the remaining three peptides (Peptides c, d and e) were joined at random. The sequence Arg-Arg was inserted between the peptides. Thus, the following six multi-epitope peptides were produced:

C.A.#1. a-Arg-Arg-b-Arg-Arg-c-Arg-Arg-d-Arg-Arg-e

C.A.#2. a-Arg-Arg-b-Arg-Arg-c-Arg-Arg-e-Arg-Arg-d

C.A.#3. a-Arg-Arg-b-Arg-Arg-d-Arg-Arg-c-Arg-Arg-e

C.A.#4. a-Arg-Arg-b-Arg-Arg-d-Arg-Arg-e-Arg-Arg-c

C.A.#5. a-Arg-Arg-b-Arg-Arg-e-Arg-Arg-c-Arg-Arg-d

C.A.#6. a-Arg-Arg-b-Arg-Arg-e-Arg-Arg-d-Arg-Arg-c

Example 7

Reactivity of the multi-epitope peptides with human IgE antibody

The six multi-epitope peptides (C.A.#1 through C.A.#6) obtained in Example 6 were dissolved in 0.2 M acetate buffer solution (pH 4.5). The solution was dispensed in quantities of 0.1 ml/well in a black plate (manufactured by Dainihon Pharmaceutical Co., Ltd.)

then allowed to stand at 4°C overnight. After the antigen solution was removed, the wells were washed three times with a washing solution and the serum (4-fold dilution) from 29 patients with cedar pollinosis and healthy subjects were each added to separate wells. The system was then reacted at 37°C for 4 hours. After the sera were removed, the wells were washed three times with a washing solution then reacted with anti-human IgE antibody (made by Pharmacia Inc.) at room temperature overnight. After washing three times with a washing solution, a substrate solution containing 0.1 mM 4-methylumbelliferyl-β-D-galacto-pyranoside/0.01 M phosphate buffer (pH 7.0), 0.1 M NaCl, 1 mM $MgCl_2$, 0.1% NaN_3 and 0.1% BSA was added, and the solution was incubated at 37°C for 2 hours. A solution of 0.1 M glycine/NaOH (pH 10.3) was added to the wells to terminate the reaction. Fluorescent intensity was measured using a fluorophotometer (Labsystems). For positive control to each multi-epitope peptide, biotin-labeled rabbit anti-d epitope IgG and peroxidase-labeled streptoavidin (made by Pierce Inc.) were reacted.

As a result, all sera from the 29 human subjects exhibited a fluorescent intensity of 3 to 5 to all of the six multi-epitope peptides (C.A.#1 through #6) (blank: 3 or 4). In contrast, when the antigen Cry j 1 extracted and purified from cedar pollen was used, a fluorescent intensity of 1,000 or more was noted in six subjects, 100 or more in 14 subjects, 10 or more in four subjects and nine or less in five subjects. In contrast, rabbit anti-d epitope peptide IgG exhibited a fluorescent intensity of 3,000 or more in response

plate was incubated for 6 days. After 0.5μCi [^{3}H] thymidine was added to the medium, incubation was continued for a further 16 hours. After the cells were harvested on a glass filter using a cell harvester, the level of [^{3}H] thymidine taken up into the cells was determined using a liquid scintillation counter.

The peripheral lymphocytes from five out of the six patients showed proliferation response to the multi-epitope peptide. The peripheral lymphocytes from one patient and two healthy subjects showed no proliferation response (Fig. 10).

The proliferation response of peripheral lymphocytes began to occur with stimulation of 0.1μg/ml of the multi-epitope peptide and increased dose-dependently. Based on the results, the concentration of the multi-epitope peptide required for inducing T cell proliferation response in vitro is at least 10μg/ml.

Peripheral lymphocytes from 17 patients with cedar pollinosis and two healthy subjects were stimulated by 10μg/ml of the multi-epitope peptide to evaluate T cell response. No response to T cell proliferation was observed with the peripheral lymphocytes from the healthy subjects. In the 17 patients, a maximum [^{3}H] thymidine uptake of 9,652 cpm was observed. When [^{3}H] thymidine uptake of peripheral lymphocytes without antigen stimulation is regarded as 1, the uptake of [^{3}H] thymidine by peripheral lymphocytes in the presence of an antigen is expressed by a stimulation index (SI). The results are shown in Fig. 11. Upon identification of T cell epitopes, SI > 2 is regarded to be positive. Similarly, SI >

subcutaneous injection of 100μg Cry j 1 together with Alum adjuvant. Ten days later, the lymphocytes were isolated to pool them as the lymphocytes from the control group and as the lymphocytes from the Cry j 1-administered mice. Cry j 1 was added to the pooled lymphocytes in doses of 0, 50 and 150μg/ml. Incubation was performed for 3 days to collect the culture supernatant. IL-2 contained in the supernatant was measured with a device manufactured by Endogen Inc. The results are shown in Fig. 12. In the control (PBS-administered) mouse group, IL-2 production increased as the concentration of Cry j 1 increased from 0 to 50 and 150μg/ml. In contrast, in the Cry j 1- administered mouse group, IL-2 production was obviously reduced, as compared to the control group, indicating that immune tolerance was acquired by administration of the cedar pollen allergen. The results verify that currently implemented hyposensitization therapy using the cedar pollen allergen is efficacious.

Example 11

Identifying T cell epitopes in CBF1 mice

Eight-week-old male CBF1 mice were boosted (i.p.) three times with 10μg of recombinant Cry j 2 (rCry j 2) at intervals of two weeks together with an adjuvant (Imject Alum, manufactured by Pierce Inc.). One week after the final booster, the spleen cells were collected from three mice and mixed together. The spleen cells (5×10^6 cells) were cultured together with each of the 74 kinds of Cry j 2 overlapping peptides (0.115μM) consisting of 15 residues in 0.2 ml RPMI medium (supplemented with 10% FCS, 2 mM L-glutamine, 50 U/ml penicillin and

Multi-epitope peptide containing T cell epitopes derived from cedar pollen and *hinoki* pollen allergens

Two peptides (Cha o 1 #8-Cry j 1 #22 core, Cha o 1 #32-Cry j 1 #22 core) were synthesized by joining Peptide No. 8 (p71-90: IFSKNLNIKLNMPLYIAGNK), which is a T cell epitope of *hinoki* pollen allergen Cha o 1 (Japanese Patent Application No. Hei 8-153527), or Peptide No. 32 (p311-330: SSGKNEGTNIYNNNEAFKVE) to Cry j 1 #22 core sequence "FIKRVSNVI" obtained in Example 14 using a peptide synthesizer (PSSM-8, Shimadzu Seisakusho Ltd.). An RR sequence was inserted between Cha o 1 #8 and Cry j 1 #22 core and between Cha o 1 #32 and Cry j 1 #22 core, that is, Cha o 1 #8 - Cry j 1 #22 core (SEQ NO: 4) and Cha o 1 #32 - Cry j 1 #22 core (SEQ NO: 5).

A Cry j 1-specific T cell line and a Cha o 1-specific T cell line were prepared from the patients with cedar pollinosis and *hinoki* pollinosis, respectively. The Cry j 1-specific T cell line and Cha o 1-specific T cell line react with neither the tubercle bacillus antigen (PPD) nor the hemolytic streptococcus cell wall (SCW) antigen. The Cry j 1-specific T cell line reacts with Cry j 1 #22 or Cry j 1 #22 core but does not react with Cha o 1 #8 or with Cha o 1 #32. The Cha o 1-specific T cell line reacts with Cha o 1 # 8 and #32 but does not react with Cry j 1 #22 or Cry j 1 #22 core (Fig. 16). However, these T cell lines all react with the multi-epitope peptide of SEQ NO: 4 and with the multi-epitope peptide of SEQ NO: 5. These results reveal that the multi-epitope peptides prepared by joining T cell epitopes derived from cedar pollen and *hinoki* pollen allergens